PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

10/531059

Applicant's or agent's file reference I 10003 PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/11436	International filing date *(day/month/year)* 15.10.2003	Priority date *(day/month/year)* 15.10.2002
International Patent Classification (IPC) or both national classification and IPC C07K14/315		
Applicant INTERCELL AG et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand 22.04.2004	Date of completion of this report 01.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Kalsner, I Telephone No. +49 89 2399-8708

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/11436

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-100 as originally filed

Sequence listings part of the description, Pages

1-70 as originally filed

Claims, Numbers

1-37 received on 25.02.2005 with letter of 25.02.2005

Drawings, Sheets

1/47-47/47 as originally filed

2. With regard to the **language,** all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 25-30, 32-35

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 25-27, 29, 30, 32-35 are so unclear that no meaningful opinion could be formed *(specify)*:

   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 28

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	9-11, 16, 17, 19, 20
	No:	Claims	1-8, 12-15, 18, 21-24, 36, 37
Inventive step (IS)	Yes:	Claims	
	No:	Claims	9-11, 16, 17, 19, 20
Industrial applicability (IA)	Yes:	Claims	1-24, 31, 36, 37
	No:	Claims	

2. Citations and explanations

see separate sheet

Ad Section III: Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 25-27, 29, 30 and 32-35 do not meet the requirements of Art. 5 and 6 PCT to such an extent that examination of these claims is not possible.

Claims 1-8 and 12-15, which the claims refer to, comprise an uncountable number of undefined sequences, which are even not limited by any functional features. Consequently, a method for identifying an antagonist to such undefined peptide (fragment) cannot be considered clear. Moreover, there are no examples in the description which would guide the skilled person to perform such method. **Claims 25-27** are therefore objected under Art. 5 and 6 PCT.

A similar objection is raised with respect to **claims 29 and 30** which are directed to a process for *in vitro* diagnosing of a bacterial infection or a disease related to expression of the polypeptide of claims 12-15. Neither the disease to be diagnosed nor the nucleic acid molecule or polypeptide whose presence has to be determined is defined in any way, thus rendering the claims completely unclear.

Likewise, **claims 32-35** are objected to under Art. 5 and 6 PCT as they all seem to refer to the same undefined polypeptides and nucleic acid (fragments).

It should be noted, in addition, that several claims refer to polypeptide according to any of the "preceding claims". The "preceding claims", however, refer to various subject-matter (other than polypeptides or nucleic acid molecules), thus rendering the claims unclear.

Ad Section IV: Lack of unity of invention

An international application must relate to one invention only or to a group of inventions so linked as to form a single general inventive concept.

Unity of invention is fulfilled only when there is a technical relationship among the inventions involving one or more of the same special technical features, special technical features being such features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

An objection under Rule 13.1, 13.2 PCT has been raised by the ISA. A

corresponding objection is raised by the IPEA.

The following documents are referred to:

D1...Meehan et al. Microbiology 144: 993-1003

The present application relates to various nucleic acid molecules encoding fibrinogen binding polypeptides and adhesion factors from Streptococcus species (SEQ ID NO: 1-10).

The technical relationship linking together the nucleotide sequences can be seen in the fact that they all encode fibrinogen binding proteins or adhesion factors of Streptococcus species. Fibrinogen binding proteins from Streptococcus species are known in the state of the art. Moreover, it is known that virulence of Streptococcus is tightly linked with fibrinogen binding proteins or adhesion factors. This concept/relationship, therefore, cannot be accepted to constitute a special technical feature as defined above as it does not define a contribution which each of the different claimed inventions, considered as a whole, makes over the prior art.

Thus, the presently claimed subject-matter falls apart in the following groups of inventions which are not unitarian (Rule 13.1, 13.2 PCT):

Invention 1: Claims 1, 7, 14, 15 (completely); 3-6, 8-13, 16-27, 29-37 (partially):

An isolated nucleic acid molecule, encoding a fibrinogen binding protein, comprising a nucleic acid having at least 70% identity to a nucleic acid sequence of SEQ ID NO: 1, 2, 3, 4, 5, or 6; an isolated nucleic acid molecule encoding a polypeptide comprising the amino acid motive of SEQ ID NO. 222, a polypeptide comprising the amino acid sequence selected from SEQ ID NO. 113-205 or SEQ ID NO. 222; process for producing such polypeptides, pharmaceutical compositions comprising the polypeptides, antibody, use of the polypeptide or antibody; methods for identifying an antagonist, process for in vitro diagnosing, affinity device comprising such polypeptide; uses of the polypeptides

Invention 2: Claims 2, 3-6, 8-13, 16-27, 29-37 (all partially):

An isolated nucleic acid molecule encoding an adhesion factor comprising a nucleic acid having at least 70% identity to a nucleic acid sequence of SEQ ID NO: 7; a polypeptide encoded by such nucleic acid molecule; process for producing such polypeptides, pharmaceutical compositions comprising the polypeptides, antibody, use of the polypeptide or antibody; methods for identifying an antagonist, process for in vitro diagnosing, affinity device comprising such polypeptide; uses of the polypeptides

Inventions 3, 4 and 5: Claims 2, 3-6, 8-13, 16-27, 29-37 (all partially)

as above, but with respect to SEQ ID NO: 8, 9 and 10, respectively

Sequences SEQ ID NO. 1-6 are variants of the same protein derived from different species of *Streptococcus agalactiae*. They are closely related in that they all encode polypeptides which comprise the repetitive motive as shown in SEQ ID NO. 222. Hence, they can be considered as one single invention.

SEQ ID NO:7-10, however, neither contain such common motive nor seem they to be related by other structural features. Thus, claims relating to these nucleic acid molecules have to be treated as individual inventions each invention relating to one single nucleic acid molecule.

As the required fees were paid this international preliminary examination report is established for the whole application.

Ad Section V: Reasoned statement with regard to novelty, inventive step or industrial applicability

1) The amendments filed with the letter dated 25 February 2005 are allowable under Art. 34(2)(b) PCT.

2) **Documents**

D1...Meehan et al. Microbiology 144: 993-1003
D2...WO 00 06736
D3...Glaser et al. (2002) Mol. Microbiol. 45: 1499-1513

D4...Osaki et al. (2002) J. Bacteriology 184: 971-982

D2 discloses Group B Streptococcus proteins and their use in vaccines. Sequences of the nucleic acids are listed in Fig. 1. Clone 15 shows 93% identity in a 540 nt overlap with SEQ ID NO: 1 of the present application. Furthermore, this clone comprises the common motif as shown in a general form in SEQ ID NO: 222.

D3 discloses the genom sequence of *S. agalactiae*. Various proteins are characterised one of which is identified as a surface protein gbs1087 containing 16 repeats of the motif LERRQRDAENR/KSQGNV.

D4 discloses *S. suis* genes encoding proteins homologous to sortase of gram-positive bacteria.

3) Novelty and inventive step

The present application relates to nucleic acid molecules encoding fibrinogen-binding polypeptides and adhesion factors.

3.1) Independent **claim 1** as well as dependent **claims 3-6 and 8** cannot be considered novel in view of the disclosure of D2. Despite the marginal amendment of claim 1 novelty could not be restored as the nucleic acid of D1 (clone 15) is still considered to "anneal under stringent hybridization conditions" or to "hybridise" to the nucleic acids of SEQ ID NO: 1-6.

3.2) Contrary to the opinion expressed by the applicant D4 does disclose isolated nucleic acid molecules. Streptococcus suis ORF203 (Acc. No. AB066354) is considered novelty destroying for **claim 2** (and consequently for **claims 3-6 and 8**) insofar as the subject-matter relates to SEQ ID NO: 8.

3.3) Likewise, the polypeptides as claimed in **claims 12-15** as well as the pharmaceutical composition of **claim 18** lack novelty over D2 and D4.

3.5) **Claim 7** is directed to an isolated nucleic acid molecule encoding for a polypeptide whereby the polypeptide comprises the amino acid motif of SEQ ID NO: 222. D3 discloses a protein gbs1087 (410 aa long) which contains **16 continuous copies** of the motif LERRQRDAENR/KSQGNV. Obviously the three amino acids GNV

(which are shown at the C-terminal end of the motif) can also be seen to precede the sequence LERR... at its N-terminus. HenceD3 is novelty destroying for claim 7.

3.4) **Claims 21 and 22** are directed to an antibody which specifically binds to the polypeptide according to claims 12-15. Claims 12-15 relate to polypeptides **comprising** a specific sequence. Hence, these polypeptides are composed of more than just the specific sequence indicated. Due to this claim language it is not clear to which part the antibody should bind. **Claims 21 and 22** thus encompass also known antibodies to known proteins which could be comprised in the polypeptides of claims 12-15.

Claims 23, 24, 31, 36 and 37 lack novelty following the same line of argumentation.

Claims 9-11 do not meet the requirements of Art. 33(3) PCT as providing vectors and cells comprising known nucleic acid molecules cannot be considered to involve an inventive step. The same argument, mutatis mutandis, holds for **claims 16-20.**

New claims 1 to 37

1. An isolated nucleic acid molecule, preferably encoding a fibrinogen-binding-polypeptide, comprising a nucleic acid sequence which is selected from the group comprising

a) a nucleic acid having at least 70% identity to a nucleic acid sequence which is selected from the group comprising SEQ ID NO 1 to SEQ ID NO 6.

b) a nucleic acid which is essentially complementary to the nucleic acid of a),

c) a nucleic acid which anneals under stringent hybridisation conditions to the polynucleotide of a) or b)and

d) a nucleic acid which, but for the degeneracy of the genetic code, would hybridize to the nucleic acid defined in a), b) or c).

2. An isolated nucleic acid molecule, preferably encoding an adhesion factor or a fragment thereof, comprising a nucleic acid sequence which is selected from the group comprising

a) a nucleic acid having at least 70% identity to a nucleic acid sequence set forth in SeqID NO 7, SeqID NO 8, SeqID NO 9 or SeqID NO 10.

b) a nucleic acid which is essentially complementary to the nucleic acid of a),

c) a nucleic acid comprising at least 15 sequential bases of the nucleic acid of a) or b),

d) a nucleic acid which anneals under stringent hybridisation conditions to the nucleic acid of a), b) or c) and

e) a nucleic acid which, but for the degeneracy of the genetic code, would hybridize to the nucleic acid defined in a), b), c) or d).

3. The isolated nucleic acid molecule according to claim 1 or 2, whereby the identity is at least 80 %, preferably at least 90 %, more preferably 100 %.

4. The isolated nucleic acid molecule according to claim 1 or 3, whereby the nucleic acid molecule encodes a fibrinogen-binding-protein comprising at least one repeat of an amino acid motive comprising 16 amino acids.

5. The isolated nucleic acid molecule according to claim 4, whereby the encoded fibrinogen-binding protein comprises 19 repeats of the amino acid motive whereby the amino acid motive is the one specified in any of claims 7 and 15.

6. The isolated nucleic acid molecule according to claims 2 or 3, whereby the nucleic acid molecule encodes an adhesion factor which interacts with epithelial cells, preferably human epithelial cells.

7. An isolated nucleic acid molecule encoding for a polypeptide whereby the polypeptide comprises an amino acid motive, whereby the amino acid motive is G-N/S/T-V-L-A/E/M/Q-R-R-X-K/R/W-A/D/E/N/Q-A/F/I/L/V/Y-X-X-K/R-X-X (SEQ ID NO 222).

8. The nucleic acid according to any of claims 1 to 7, wherein the nucleic acid is DNA, RNA or mixtures thereof, preferably the nucleic acid molecule is isolated from a genomic DNA.

9. A vector comprising a nucleic acid molecule according to any of claims 1 to 8.

10. The vector according to claim 8, wherein the vector is adapted for recombinant expression of the polypeptide encoded by any of the nucleic acid molecules according to any of claims 1 to 8.

11. A cell, preferably a host cell, comprising the vector according to claim 9 or 10.

12. A polypeptide, preferably a fibrinogen-binding-polypeptide and/or an adhesion factor, comprising an amino acid sequence, whereby the amino acid sequence is encoded by a nucleic acid molecule according to any one of claims 1 to 8, and fragments of said polypeptide.

13. A polypeptide, preferably a fibrinogen-binding-polypeptide and/or an adhesion factor, comprising an amino acid sequence, whereby the amino acid sequence is selected from the group comprising Seq ID NO 11 to 20.

14. A polypeptide, preferably a fibrinogen-binding-polypeptide and/or an adhesion factor, comprising an amino acid sequence, whereby the amino acid sequence is selected from the group comprising Seq ID NO 113 to 205.

15. A polypeptide, preferably a fibrinogen-binding-polypeptide and/or an adhesion factor, comprising an amino acid motive, whereby the polypeptide comprises an amino acid motive, whereby the amino acid motive is G-N/S/T-V-L-A/E/M/Q-R-R-X-K/R/W-A/D/E/N/Q-A/F/I/L/V/Y-X-X-K/R-X-X (SEQ ID NO 222).

16. A process for producing a polypeptide according to any of claims 12 to 15 or a fragment thereof, comprising expressing the nucleic acid molecule according to any of claims 1 to 8.

17. A process for producing a cell which expresses a polypeptide according to any of claims 12 to 15 or a fragment thereof, comprising transforming or transfecting a suitable host cell with the vector according to claim 9 or 10.

18. A pharmaceutical composition, especially a vaccine, comprising a polypeptide or a fragment thereof, as defined in any one of claims 12 to 15 or a nucleic acid molecule according to any of claims 1 to 8.

19. The pharmaceutical composition according to claim 18, characterized in that it comprises an immunostimulatory substance, whereby the immunostimulatory substance is preferably selected from the group comprising polycationic polymers,

immunostimulatory deoxynucleotides (ODNs), synthetic KLK peptides, neuroactive compounds, alumn, Freund's complete or incomplete adjuvants or combinations thereof.

20. Use of a polypeptide according to any one of the claims 12 to 15 or a fragment thereof for the manufacture of a medicament, especially for the manufacture of a vaccine against bacterial infection.

21. An antibody, or at least an effective part thereof, which specifically binds to the polypeptide according to claims 12 to 15.

22. The antibody according to claim 21, wherein the antibody is selected from the group comprising monoclonal antibodies, polyclonal antibodies, chimeric antibodies, humanized antibodies and fragments of each thereof.

23. Use of a polypeptide according to any of the claims 12 to 15, for the manufacture of an antibody.

24. Use of the antibody according to claim 21 or 22 for the preparation of a medicament for treating or preventing bacterial infections, especially *Streptococcus agalactiae* infections.

25. A method for identifying an antagonist capable of reducing or inhibiting the activity of the polypeptide or fragment thereof according to any of the claims 12 to 15 or which is capable of binding to the polypeptide according to any of claims 12 to 15 comprising:

a) contacting an isolated or immobilized polypeptide according to any of the claims 12 - 15 or a fragment thereof with a candidate antagonist under conditions to permit binding of said candidate antagonist to said polypeptide or fragment thereof, in the presence of a component capable of providing a detectable signal in response to the binding of the candidate antagonist to said polypeptide or fragment thereof; and

b) detecting the presence or absence of a signal generated in response to the binding of the antagonist to the polypeptide or fragment thereof, preferably the presence of a signal indicating a compound capable of inhibiting or reducing the activity of the polypeptide or fragment thereof.

26. A method for identifying an antagonist capable of reducing or inhibiting the activity of a polypeptide or a fragment thereof according to any of claims 12 to 15 comprising:

a) providing the polypeptide according to any of the claims 12 to 15 or a fragment thereof,

b) providing an interaction partner of the polypeptide according to any of the claims 12 to 15, preferably an antibody according to claim 21 or 22.

c) providing a candidate antagonist,

d) reacting the polypeptide, the interaction partner of the polypeptide and the candidate antagonist, and

e) determining whether the candidate antagonist inhibits or reduces the activity of the polypeptide.

27. A method for identifying an antagonist capable of reducing or inhibiting the interaction activity of the polypeptide according to any of claims 12 to 15 or a fragment thereof to its interaction partner comprising:

a) providing the polypeptide according to any of claims 12 to 15 or a fragment thereof,

b) providing an interaction partner to said polypeptide or a fragment thereof, preferably an antibody according to claim 21 or 22,

c) allowing interaction of said polypeptide or fragment thereof to said interaction partner to form an interaction complex,

d) providing a candidate antagonist,

e) allowing a competition reaction to occur between the candidate antagonist and the interaction complex, and

f) determining whether the candidate antagonist inhibits or reduces the interaction activities of the polypeptide or the fragment thereof with the interaction partner.

28. An antagonist identified or identifiable by a method according to claim 26 or 27.

29. A process for *in vitro* diagnosis of a bacterial infection, preferably *Streptococcus agalactiae* infection, comprising the step of determining the presence of a nucleic acid molecule according to any of the preceding claims, or of a polypeptide according to any of the preceding claims.

30. A process for *in vitro* diagnosing a disease related to expression of the polypeptide according to any of claims 12 to 15 or a fragment thereof, comprising determining the presence of a nucleic acid sequence encoding said polypeptide or a fragment thereof according to any of claims 1 to 8, or the presence of the polypeptide according to any of claims 12 to 15 or a fragment thereof.

31. An affinity device comprising a support material and immobilized to said support material a polypeptide according to any of the preceding claims or a nucleic acid molecule according to any of the preceding claims.

32. Use of a polypeptide according to any of the preceding claims or a fragment thereof for the isolation and/or purification and/or identification of an interaction partner of said polypeptide or a fragment thereof.

33. Use of any of the polypeptides according to any of the preceding claims for the generation of a peptide binding to said polypeptide.

34. The use according to claim 33, whereby the peptide is selected from the group comprising anticalines.

35. Use of a polypeptide according to any of the preceding claims for the manufacture or generation of a functional nucleic acid, whereby the functional nucleic acid is selected from the group comprising aptamers and spiegelmers.

36. Use of a polypeptide according to any of the preceding claims as an antigen.

37. Use of a nucleic acid according to any of claims 1 to 8, for the manufacture or generation of a functional ribonucleic acid, wherein the functional ribonucleic acid is selected from the group comprising ribozymes, antisense nucleic acids and siRNA.